FEBS Letters 583 (2009) 320–324

FEBS Letters

journal homepage: www.FEBSLetters.org

Atomic structure of mutant PPARγ LBD complexed with 15d-PGJ$_2$: Novel modulation mechanism of PPARγ/RXRα function by covalently bound ligands

Tsuyoshi Waku, Takuma Shiraki, Takuji Oyama, Kosuke Morikawa *

The Takara-Bio Endowed Division, Department of Biomolecular Recognition, Institute for Protein Research, Osaka University, Open Laboratories of Advanced Bioscience and Biotechnology, 6-2-3, Furuedai, Suita, Osaka 565-0874, Japan

ARTICLE INFO

Article history:
Received 20 November 2008
Revised 5 December 2008
Accepted 5 December 2008
Available online 26 December 2008

Edited by Hans Eklund

Keywords:
PPARγ
covalently bound ligand
PPARγ/RXRα
activating process
modulation mechanism
Crystal structure

ABSTRACT

15-deoxy-Δ^{12,14}-prostaglandin J$_2$ (15d-PGJ$_2$) activates a nuclear receptor heterodimer, peroxisome proliferators-activated receptor γ (PPARγ)/ retinoid X receptor (RXRα) through covalent binding to Cys285 in PPARγ ligand-binding domain (LBD). Here, we present the 1.9 Å crystal structure of C285S mutant LBD complexed with 15d-PGJ$_2$, corresponding to the non-covalently bound state. The ligand lies adjacent to a hydrogen-bond network around the helix H2 and the nearby β-sheet. Comparisons with previous structures clarified the relationships between PPARγ function and conformational alterations of LBD during the process of covalently binding ligands, such as 15d-PGJ$_2$, and thus suggested a mechanism, by which these ligands modulate PPARγ/RXRα function through conformational changes of the loop following helix H2′ and the β-sheet.

1. Introduction

Peroxisome proliferators-activated receptor γ (PPARγ), which belongs to a nuclear receptor superfamily, functions as obligate heterodimers with retinoid X receptors (RXRs) [1,2]. This receptor is particularly noteworthy, because it participates in various biological phenomena, such as insulin sensitization, adipogenesis, atherosclerosis, inflammation, and carcinogenesis [1,3–7].

PPARγ is activated by physiological fatty-acid derivatives, including nitrated or oxidized fatty acids [8–13]. Several polyunsaturated lipids, such as 15-deoxy-Δ^{12,14}-prostaglandin J$_2$ (15d-PGJ$_2$), activate the receptor-mediated transcription by covalently binding to a unique cysteine (Cys285) in the ligand-binding domain (LBD) [14,15]. Our recent study revealed the following facts [16]; (1) the covalent binding to endogenous fatty acids, such as 15d-PGJ$_2$, results in the structural alteration of the loop following helix H2′ and in the rearrangement of the side-chain network around Cys285 within LBD; (2) the conformational difference of the loop provides the ligand-type specific effects in term of the distinct degree of the receptor activity; (3) Phe287, one of these repositioned residues, has an important role in receptor activation. However, it has been unclear how the conformational transmission by non-covalent binding to these ligands modulates receptor function before covalent bond formation, since the atomic details of the non-covalently bound state were unavailable.

In this study, we determined the 1.9Å crystal structure of C285S mutant LBD, which is bound to 15d-PGJ$_2$ in the covalent modification-independent manner. The ligand mainly interacts with the residues on helices H3, H5, and H12, and it is located in the vicinity of the network of water molecules formed around the helix H2 and the nearby β-sheet. Comparison of crystal structures of the three distinct binding states to 15d-PGJ$_2$ [16] showed that the conformation of the loop following helix H2′ changes by non-covalent binding, and subsequently the side-chain networks around Cys285, particularly Phe287, are rearranged by covalent binding. This result indicated that covalently bound ligands might sequentially direct the ligand-type specific effects and the switching-on of PPARγ activation, through the two distinct structural alterations. Furthermore, the superposition of the present structure on the crystal structure of intact PPARγ/RXRα complexed with DNA [17] suggested intriguing roles of the 15d-PGJ$_2$ binding pocket in coactivator recruitment and interactions with the DNA-binding domain (DBD) of RXRα.

Abbreviations: PPARγ, peroxisome proliferators-activated receptor γ; RXR, retinoid X receptor; 15d-PGJ$_2$, 15-deoxy-Δ^{12,14}-prostaglandin J$_2$; LBD, ligand-binding domain; LBP, ligand-binding pocket; DBD, DNA-binding domain

* Corresponding author. Fax: +81 6 6872 8219.
E-mail address: morikako@protein.osaka-u.ac.jp (K. Morikawa).

0014-5793/$34.00
doi:10.1016/j.febslet.2008.12.017

2. Materials and methods

2.1. Protein preparation

The plasmids and recombinant proteins of the wild type and C285S mutant human PPARγ LBDs (hereafter referred to as wtLBD and mtLBD, respectively), were prepared as described previously [15,16]. Using UV absorption spectral analyses [15], we confirmed that the mtLBD did not bind covalently to 15d-PGJ_2 (data not shown).

2.2. Crystallization, data collection, and model refinement

To avoid variability derived from crystallization conditions and different crystal packing, we first crystallized the mtLBD without the ligand, under the conditions reported previously [16], and then soaked the crystals with 15d-PGJ_2. The diffraction data were collected at BL38B1 in SPring-8 (Harima, Japan), as described previously [16]. All data were processed using HKL2000 [18]. The structure was solved by the molecular replacement method, using the wtLBD covalently bound to 15d-PGJ_2 (PDB ID 2ZK1) as a search model [16]. The model refinement was carried out using CNS and O [19,20]. The crystallographic data and refinement statistics are summarized in Supplementary Table 1. All structural figures were made with Chimera [21]. Since helix H12 in monomer B was frequently disoriented, depending upon the crystal packing, we omitted monomer B from the figure. Coordinates and structural factors have been deposited in the Protein Data Bank (PDB ID 2ZVT).

3. Results

3.1. Structure of C285S mutant PPARγ LBD complexed with 15d-PGJ_2

The structure of mtLBD complexed with 15d-PGJ_2 was refined at 1.9Å resolution (Fig. 1A). The final model of the complex presented the clear density of 15d-PGJ_2 (Fig. 1B). This fatty-acid ligand was found to interact mainly with helices H3, H5, and H12, and was located in the close vicinity of the network of water molecules that adjoins helix H2 and the nearby β-sheet consisting of β2 and β3. Notably, most of the water molecules within ligand-binding pocket (LBP) are involved in this network (Fig. 1C and D). This result indicates that the LBP is divided into hydrophobic and hydrophilic regions. Hydrogen/deuterium exchange mass spectrometry experiments revealed that amino acid residues, facing this hydrogen-bond network, generally exhibit higher exchange rates than the other residues [17,19]. The clustering of water molecules may play some role in enhancing the hydrogen/deuterium exchange rates.

3.2. Structural differences among distinct 15d-PGJ_2-binding states of PPARγLBD

To clarify the structure-function relationships of the state with non-covalently bound 15d-PGJ_2, we compared the previously published crystal structures of the unliganded wtLBD (PDB ID 2ZK0) and the wtLBD covalently bound to 15d-PGJ_2 (PDB ID 2ZK1) [16] with the present structure. We first focused on the position and

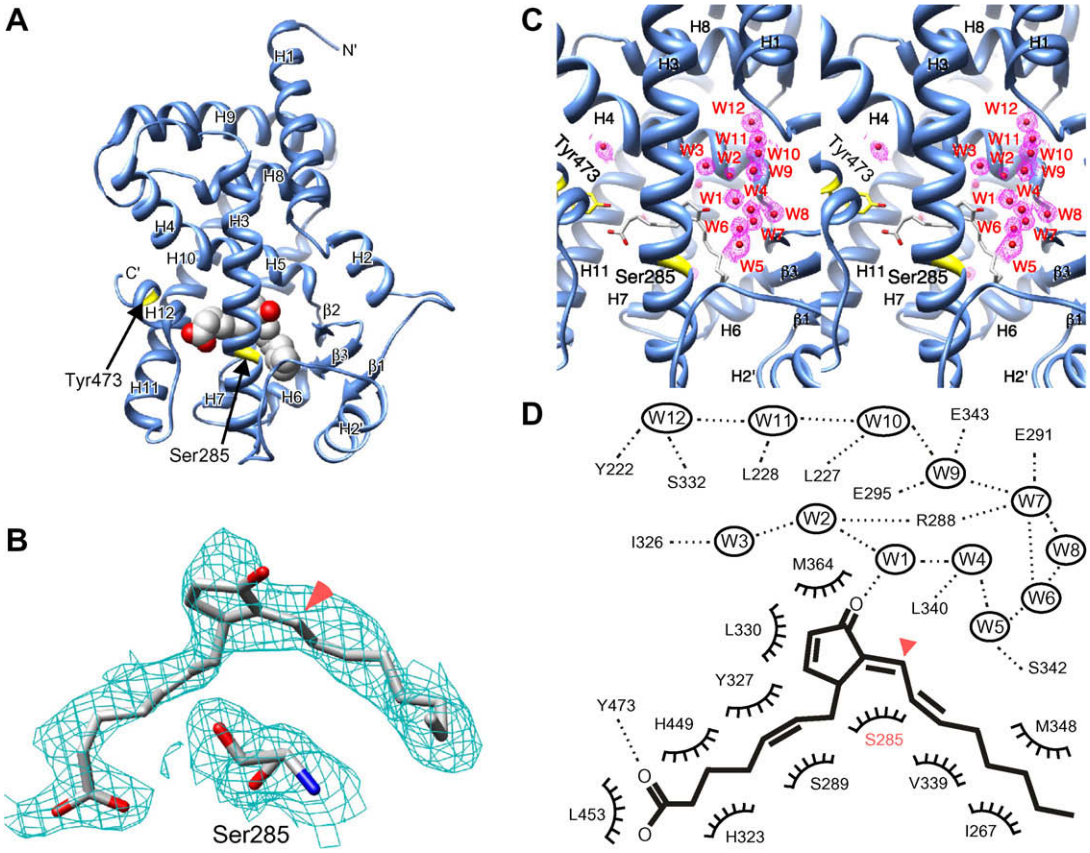

Fig. 1. Structure of C285S mutant PPARγ LBD complexed with 15d-PGJ_2. (A) Overall structure. The Cα atoms of mtLBD are modeled as blue ribbons. The non-covalently bound ligand is depicted as a space-filling model. (B) The composite omit 2|Fo|-|Fc| electron density map of 15d-PGJ_2 (contoured at 1σ). The ligand and the Ser285 residue are represented as stick models. The arrowhead indicates a carbon atom that is potentially covalently bound to Cys285. (C) Close-up stereo view of LBP. Water molecules in LBP are represented as spheres, with the composite omit 2|Fo|-|Fc| electron density map contoured at 1.0σ. (D) Schematic representation of the interaction between mtLBD and 15d-PGJ_2. The ligand is shown by black lines. Hydrophobic interactions are indicated by arcs, and hydrogen bonds are depicted by dashed lines. Ser285 is colored by red. Key water molecules are represented by "w", and are numbered.

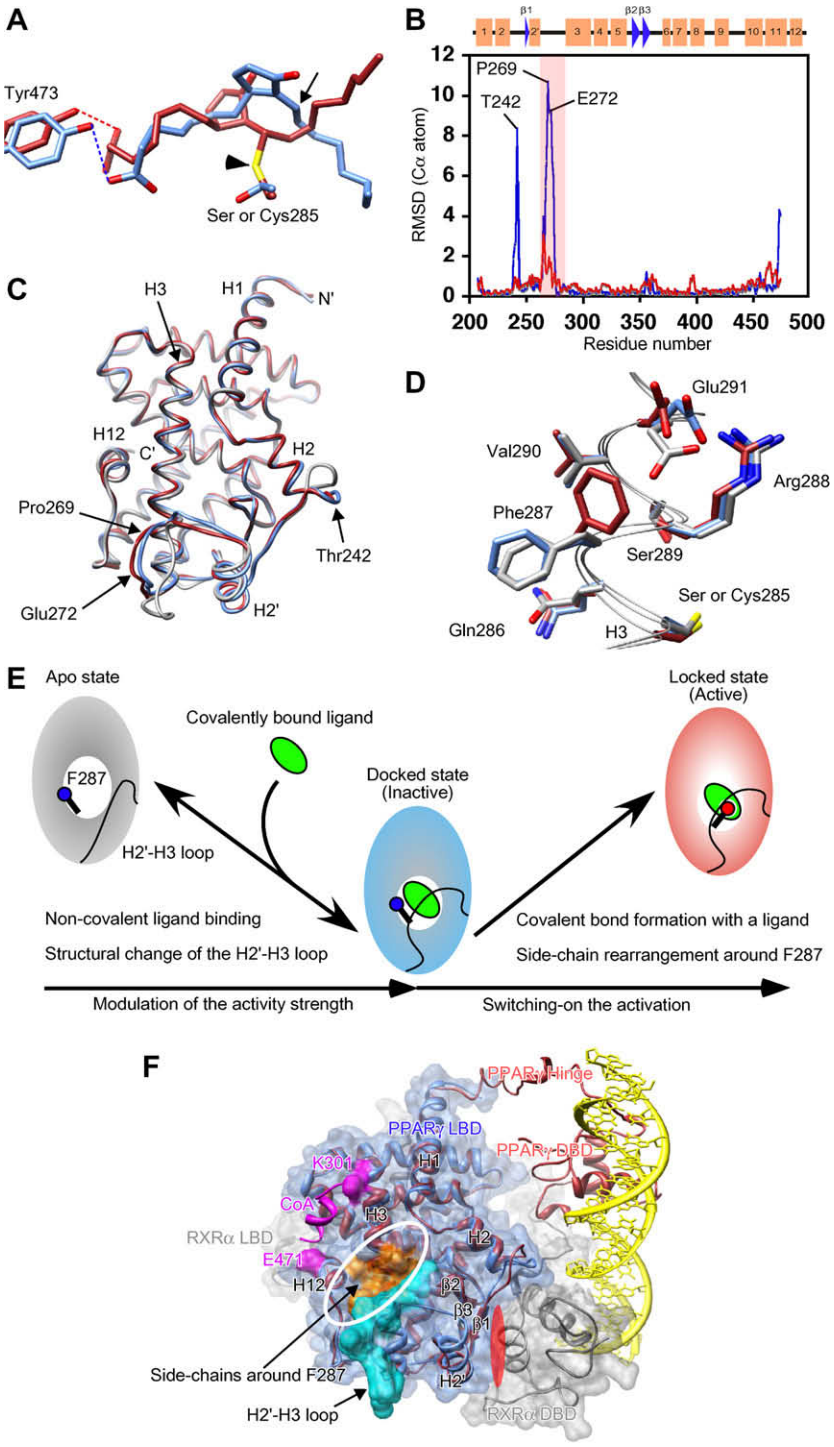

Fig. 2. Comparisons among various PPARγ structures. (A) Superposition of 15d-PGJ_2 in the non-covalently bound (blue) and covalently bound (red) states. Hydrogen bonds between the ligand and Tyr473 are indicated by dashed lines in blue (non-covalently bound state) and red (covalently bound state). The arrowhead indicates the covalent bond between the ligand and Cys285 in wtLBD. The arrow indicates the carbon atoms that are potentially covalently bound to the cysteine. (B) Root mean square deviation (RMSD) of the Cα atoms, calculated between the unliganded and non-covalently bound states (blue line) and between the non-covalently and covalently bound states (red line). The red area represents the loop following helix H2′. Secondary structural elements are indicated at the top. The residues that move more than 8 Å are noted. (C) Superposition of LBD structures in the three states. The Cα atoms of LBDs are depicted in gray (unliganded state), blue (non-covalently bound state), and red (covalently bound state). The residues noted in (B) are mapped. (D) The side-chain orientation around Ser285 on helix H3. The side chains among the unliganded, non-covalently bound, and covalently bound states are depicted in gray, blue, and red, respectively. (E) Schematic model representing the conformational changes of PPARγ LBDs during the activating process by covalently bound ligands. The LBDs are colored in gray (apo state), blue (docked state), and red (locked state), and the ligand is colored green. The loop following helix H2′ is represented as a black line and is named the "H2′–H3 loop". Phe287 in the apo and docked states is depicted as a blue circle, whereas that in the locked state is depicted as a red circle. (F) Superposition of the crystal structures of the 15d-PGJ_2/mtLBD and the intact PPARγ/RXRα with the coactivator peptide on DNA (PDB ID 3DZY). The surfaces in the 15d-PGJ_2/mtLBD are colored as follows; the side chains around Phe287 (orange), the loop region following H2′ (cyan), and the canonical coactivator-binding interface (magenta). The white solid line encloses the putative secondary coactivator-binding interface. The red mark represents the interactive region between PPARγ LBD and RXRα DBD in the intact complex.

the orientation of 15d-PGJ_2 in LBP. The structural comparisons between the ligand/LBD complexes with and without the covalent bond formation revealed that the carboxyl group of the ligand interacts with the side chain of Tyr473 on helix H12 in each complex (Fig. 2A). This result indicated that the ligand-configurations are overall similar before and after the covalent bond formation with Cys285 in LBD. However, the structure is slightly different in atomic detail, probably due to a minor environmental change caused by the substitution of serine for Cys285. It is likely that this minor difference is alleviated by changes in the torsion angles near Cys285 during the covalent bond formation.

However, a plotting of the root mean square deviations (RMSDs) of the Cα atoms versus the residue numbers and a structural superposition revealed a striking difference in the backbone structures, confined to the loop following helix H2′, between the apo and non-covalently bound states (Fig. 2B blue line, and C). On the other hand, the covalent binding caused side-chain rearrangements around Cys285, although the backbone structure remained unchanged (Fig. 2B red line, C, and D), indicating that the covalent modification with 15d-PGJ_2 alters the local side-chain network around Cys285.

4. Discussion

4.1. Structural and functional modulation of PPARγ by non-covalent ligand binding

Our earlier study using a cell-based assay revealed that C285S mutation in PPARγ LBD abolished the transcriptional activation by 15d-PGJ_2 [15]. Using the mutant recombinant protein, we have now presented the 1.9 Å crystal structure of the mutant LBD complexed with the ligand (Fig. 1). This structure is the first atomic-detail demonstration of the non-covalently bound, inactive state before the covalent bond formation during the receptor activating process (Fig. 2A–D). We recently reported that conformational differences in the loop following helix H2′ determine the receptor-activity strength, while several residues, such as Phe287, that shift due to the covalent modification are crucial for the receptor activation by covalently bound ligands, such as 15d-PGJ_2 [16]. Taken together, the present study indicates that non-covalent binding of ligands modulates the structure of the loop in the ligand-type specific manner, and that covalent binding subsequently induces the activation by the rearrangement of the side-chain network around Phe287 (Fig. 2E); the non-covalently bound, inactive intermediate complex is designated as the "docked state", and the covalently bound and active complex corresponds to the "locked state" according to our previous classification [22].

4.2. Novel modulation of the PPARγ/RXRαactivity by covalently bound ligands

It is well known that hydrogen-bond formation between the carboxyl group of the synthetic ligand and the side chain of Tyr473 on helix H12 settles this helix into the active form, thereby generating the canonical coactivator-binding interface with Lys471 on the same helix and Glu301 on helix H3 [23–25]. Interestingly, this interaction was found in the present structure, corresponding to the non-covalently bound, inactive state (Fig. 2A). We confirmed that the Y473F mutant retains the ability to bind covalently to 15d-PGJ_2 (data not shown), but loses the ligand-induced transcriptional activity [22]. On the other hand, the C285S mutant cannot be activated through covalent modification, but accommodates the ligand into LBP [15,22]. Furthermore, our recent report indicated that the direct interaction with Tyr473 is not always essential for the transcriptional activation by covalently bound ligands [16]. Taken together, these results not only support our proposed model (Fig. 2E), but also suggest that the interaction with Tyr473 may transiently stabilize the ligand to take an adequate configuration for covalent modification with Cys285.

A recent report describing the crystal structures of the intact PPARγ/RXRα with ligands and a coactivator peptide on DNA (PDB ID 3DZY) revealed that b-strand elements S1, S2, and S3 (in this study, S1 is denoted as β1, and S2 and S3 are β2) and helix H6 in PPARγ LBD interact directly with RXRα DBD [17]. A structural comparison indicated that the loop following helix H2′ and the side chains around Phe287 are located in the close vicinity of the canonical coactivator-binding interface. We previously proposed that this region might constitute the secondary coactivator-binding interface [26]. This putative interface occupies a reasonable position even in the intact receptor complex. Intriguingly, the hydrogen-bond network and RXRα DBD are located on opposite sides to each other, across β-sheet consisting of β2 and β3 (Fig. 2F). It is possible that the covalently bound ligands perturb the hydrogen-bond network, thereby affecting the interaction between PPARγ LBD and RXRα DBD.

Acknowledgments

We thank Dr. Toshifumi Takao for helpful comments, and Drs. Kazuya Hasegawa and Seiki Baba for support in the X-ray diffraction data collection at BL38B1 in SPring-8. This study was supported by a donation from TAKARA Bio Inc. and by a Grant-in-Aid for Creative Scientific Research Program (18GS0316) from the Japan Society for the Promotion of Science (JSPS). We all declare that none of the authors have a financial interest related to this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.12.017.

References

[1] Walczak, R. and Tontonoz, P. (2002) PPARadigms and PPARadoxes: expanding roles for PPARγ in the control of lipid metabolism. J. Lipid Res. 43, 177–186.
[2] Mangelsdorf, D.J. and Evans, R.M. (1995) The RXR heterodimers and orphan receptors. Cell 83, 841–850.
[3] Rosen, E.D. and Spiegelman, B.M. (2001) PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth. J. Biol. Chem. 276, 37731–37734.
[4] Lee, C.-H., Olson, P. and Evans, R.M. (2003) Minireview: lipid metabolism, metabolic disease, and peroxisome proliferator-activated receptors. Endocrinology 144, 2201–2207.
[5] Willson, T.M., Lambert, M.H. and Kliewer, S.A. (2001) Peroxisome proliferator-activated receptor γ and metabolic disease. Annu. Rev. Biochem. 70, 341–367.
[6] Pakala, R. et al. (2004) Peroxisome proliferator-activated receptor γ: its role in metabolic syndrome. Cardiovasc. Radiat. Med. 5, 97–103.
[7] Semple, R.K., Chatterjee, V.K.K. and O'Rahilly, S. (2006) PPARγ and human metabolic disease. J. Clin. Invest. 116, 581–589.
[8] Kliewer, S.A., Lehmann, J.M. and Willson, T.M. (1999) Orphan nuclear receptors: shifting endocrinology into reverse. Science 284, 757–760.
[9] Baker, P.R.S. et al. (2005) Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. J. Biol. Chem. 280, 42464–42475.
[10] Schopfer, F.J. et al. (2005) Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor γ ligand. Proc. Natl. Acad. Sci. USA 102, 2340–2345.
[11] Li, Y. et al. (2008) Molecular recognition of nitrated fatty acids by PPARγ. Nat. Struct. Mol. Biol. 15, 865–867.
[12] Forman, B.M. et al. (1995) 15-deoxy-$D^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. Cell 83, 803–812.
[13] Kliewer, S.A. et al. (1995) A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 83, 813–819.
[14] Itoh, T. et al. (2008) Structural basis for the activation of PPARγ by oxidized fatty acids. Nat. Struct. Mol. Biol. 15, 924–931.
[15] Shiraki, T. et al. (2005) α, β-unsaturated ketone is a core moiety of natural ligands for covalent binding to peroxisome proliferator-activated receptor γ. J. Biol. Chem. 280, 14145–14153.

[16] Waku, T. et al. (2008) Structural insight into PPARγ activation through covalent modification with endogenous fatty acids. J. Mol. Biol., doi:10.1016/j.jmb.2008.10.039.
[17] Chandra, V. et al. (2008) Structural of the intact PPAR-γ–RXR-α nuclear receptor complex on DNA. Nature 456, 350–356.
[18] Otwinowski, Z. and Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode. Method Enzymol. 276, 307–326.
[19] Brunger, A.T. et al. (1998) Crystallography and NMR system: a new software suite for macromolecular structure determination. Acta Cryst. D 54, 905–921.
[20] Jones, T. et al. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Cryst. A. 47, 110–119.
[21] Pettersen, E. et al. (2004) UCSF Chimera – a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.
[22] Shiraki, T. et al. (2006) Spectroscopic analysis of the binding kinetics of 15d-PGJ_2 to the PPARγ ligand-binding domain by multi-wavelength global fitting. Biochem. J. 393, 749–755.
[23] Xu, H.E. et al. (2001) Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. USA 98, 13919–13924.
[24] Nolte, R.T. et al. (1998) Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. Nature 395, 137–143.
[25] Gampe, Jr. et al. (2000) Asymmetry in the PPARγ/RXRα crystal structure reveals the molecular basis of heterodimerization among nuclear receptors. Mol. Cell 5, 545–555.
[26] Shiraki, T. et al. (2005) Rational discovery of a novel interface for a coactivator in the peroxisome proliferator-activated receptor γ: Theoretical implications of impairment in type 2 diabetes mellitus. Proteins 58, 418–425.